DETERMINATION OF NITRITE IN WATER USING THE AQ2 OR AQ400 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. This SOP is applicable to the determination of nitrite in surface, ground and saline water, and in domestic and industrial wastes. The applicable range for this method is from the method detection limit (MDL) of 0.002 mg N/L to 0.20 mg N/L. Samples containing levels higher than 0.20 mg N/L should be diluted to be within the calibration range.

Key Parameters	
Calibration Range	0 – 0.20 mg N/L
Minimum Detection Limit (MDL)	0.002 mg N/L
Practical Quantitation Limit (PQL)	0.005 mg N/L

1.2. Tests covered by this SOP are W-NO2 and W-NO2-F.

2. SUMMARY OF THE METHOD

2.1. This method is based upon EPA method 353.2, Rev. 2.0 (1993) and Seal Analytical AQ2 or AQ400 method EPA-137-A Rev.1.

2.2. The diazonium compound, formed by diazotation of sulfanilamide by nitrite in water under acid conditions, is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to produce a reddish-purple azo dye, which is measured colorimetrically at a wavelength of 520 nm.

2.3. Interferences: Background absorbance (color) at the analytical wavelength may interfere. Residual chlorine in the sample may cause low spike recoveries – this can be removed by adding sodium thiosulfate to the sample aliquot prior to spiking (see section 5.4 in DEP SOP NU-066 for chlorine test and treatment procedures). Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation

Instrument ID	Component	Manufacturer	Model
AQ2-1	Discrete Analyzer	SEAL Analytical	AQ2
AQ400-1	Discrete Analyzer	SEAL Analytical	AQ400

3.2. Instrument Accessories

Accessory	Manufacturer/Vendor	Model/Catalog Number
Lamp Assembly (AQ400)	SEAL Analytical	6100
Lamp Assembly (AQ2)	SEAL Analytical	5100
Aspiration Probe	SEAL Analytical	5110
10 mm Cuvette Inlet Tubing (AQ400)	SEAL Analytical	5781
10 mm Cuvette Inlet Tubing (AQ2)	SEAL Analytical	5671
10 mm Cuvette Outlet Tubing (AQ400)	SEAL Analytical	5778

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10 mm Cuvette Outlet Tubing (AQ2)	SEAL Analytical	5672
Peristaltic Pump Tubing	SEAL Analytical	5145
Syringe Body, Piston, O-Ring	SEAL Analytical	5195-1
Reagents Tray (AQ400)	SEAL Analytical	MA000160
Reagents Tray (AQ2)	SEAL Analytical	MA000106S
Sample Probe (AQ400)	SEAL Analytical	6125-S
Sample Probe (AQ2)	SEAL Analytical	5125
Sample Tray (80 Position Tray – AQ400)	SEAL Analytical	9-5351
Sample Tray (57 Position Tray – AQ2)	SEAL Analytical	5350
Probe Wash Assembly	SEAL Analytical	MA000102
Or Equivalent		

3.3. Equipment

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Analytical Balance	Mettler Toledo/Fisher	MS204TS
Stirrer	Fisher Scientific	11-100-49S (Discontinued)
Stir Bars, Assorted	Fisherbrand/Fisher	14-513-61; 14-513-58; S68069
Adjustable Pipette, 10-100 µL	Eppendorf/Fisher	3123000047/13690029
Adjustable Pipette, 100-1000 µL	Eppendorf/Fisher	3123000063/13690032
Adjustable Pipette, 1-10 mL	Eppendorf/Fisher	3123000080/13690034
Filtering Flask, 1L	Pyrex/Fisher (SR)	10180F
Glass Beaker, Assorted	Pyrex/Fisher (SR)	02-540-K; 02-540L; 02-540P
Volumetric Flask, Class-A, Assorted	Pyrex/Fisher (SR)	10-210-5C; 10-210-5E; 10-210-5F; 10-210-5G; 10-210-5H
Magnetic Filter Funnel, 47 mm	Andwin Scientific/Fisher	NC0006472
Graduated Cylinder, Assorted	KIMAX/Fisher (SR)	08-548D; 08-546A
Glass Funnel	Fisher (SR)	10-346-5B
Or Equivalent		
[1]Note: (SR) designates equipment is typically ordered through the in-house stockroom		

3.4. Consumables and Supplies

Supplies/Consumables	Vendor[1]	Catalog Number
Reaction Segment	SEAL Analytical (SR)	5000
Reaction Container	SEAL Analytical	5010
2 mL Sample Cup	SEAL Analytical	6015
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-120; 08-732-122
Pipette Tips, 200 µL	Eppendorf/Fisher (SR)	05-403-41
Pipette Tips, 1000 µL	Eppendorf/Fisher (SR)	05-403-43
Pipette Tips, 1-10 mL	Eppendorf/Fisher (SR)	05-403-116
Tube, Centrifuge, 15 mL, Polypropylene	Corning/Fisher(SR)	05-538-59A
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-100; 02-202-102
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Transfer Pipet, 5 mL	Fisher (SR)	13-711-5AM
Parafilm	Fisher (SR)	13-374-12
Nitrocellulose Membrane	Fisherbrand/Fisher	SA1J792H5
Or Equivalent		
[1]Note: (SR) designates equipment is typically ordered through the in-house stockroom		

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4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Prior to using the chemicals in this analysis, please read their MSDS and the safety notes found in the safety/hazardous waste section of this SOP.

Note: All reagent and standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the Reagent and Standard Logsheet (NU-RGTSTD). This sheet needs to be updated and submitted as part of the analysis report folder. If available, a Certificate of Analysis for each purchased standard or reagent is maintained in the \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrite\CertificatesofAnalysis\ folder. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Nitrite Waste WatR	SIM NUT AMP	ERA	770	See CoA	See CoA	Room Temp
4.1.2.	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	ACS, ≥97%	See CoA	Room Temp
4.1.3.	Ethylenediamine Tetraacetic Acid	EDTA	Fisher	S311-500	Certified ACS	See CoA	Room Temp
4.1.4.	Ammonium Chloride	NH_4Cl	Fisher Chemical	A661-500	ACS, ≥99.5%	See CoA	Room Temp
4.1.5.	N-(1-Naphthy)-Ethylenediamine	NED	Acros Organics (SR)	AC42399-0250	ACS Reagent	See CoA	Room Temp
4.1.6.	o-Phosphoric Acid	H_3PO_4	Fisher (SR)	A242-212	85% Certified ACS	See CoA	Room Temp
4.1.7.	Sodium Chloride	NaCl; Salt	Fisher (SR)	S271-500	Certified ACS	See CoA	Room Temp
4.1.8.	Sulfanilamide	$C_6H_8N_2O_2S$	Acros Organics (SR)	AC132855000	0.98%	See CoA	Room Temp
4.1.9.	Chloroform	$CHCl_3$	Fisher	C298-500	Certified ACS	See CoA	Room Temp
4.1.10.	Potassium Nitrite	KNO_2	Fisher	P267-500	Certified ACS	See CoA	Room Temp
4.1.11.	Bleach, Generic	NaOCl for cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	See CoA	Fridge
4.1.12.	Deionized Water	H_2O	In-House	N/A	N/A	Daily	N/A

Or Equivalent

[1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom

4.2. Reagents

4.2.1. NED Color Reagent –

NaOH (4.1.2.)	2.0 g
EDTA (4.1.3.)	1.0 g
o-Phosphoric Acid (4.1.6.)	50 mL
Ammonium Chloride (4.1.4.)	50 g
Sulfanilamide (4.1.8.)	12.5 g

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NED (4.1.5.)	0.625 g
DI Water (4.1.12.)	to 500 mL

To a 500 mL beaker containing about 250 mL of deionized (DI) water add 2.0 g sodium hydroxide pellets and 1.0 g ethylenediamine tetraacetic acid disodium salt hydrate (EDTA). Stir to dissolve completely. Add 50 mL of concentrated phosphoric acid. **Caution**: The beaker will become warm! While stirring add 50 g ammonium chloride, 12.5 g sulfanilamide and additional DI water to bring the volume to about 450 mL. Dissolve completely. Add 0.625 g N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) and stir to dissolve completely. Dilute to 500 mL.

Filter the solution using a 0.45 micron filter and transfer to a dark container.

Store in the refrigerator. Holding time, 30 days. Discard if it develops a significant pink color.

4.2.2. Alkaline EDTA Rinse (Cuvette Wash) –

EDTA (4.1.3.)	5 g
NaOH (4.1.2.)	10 g
DI Water (4.1.12.)	to 500 mL

Add approximately 250 mL DI water to a 500 mL volumetric flask. Add 5 g sodium EDTA and 10 g NaOH to the flask and swirl until the NaOH dissolves. Dilute to volume with DI water and mix well.

Holding time, 60 days.

4.3. Standards

4.3.1. QCNI, a second source QC standard, should be prepared from a stock from a different manufacturer or a different lot than the calibration standards. A certified standard, such as the ERA SIM NUT (4.1.1.), is preferred. A certified standard should be diluted with DI water if needed to bring the concentration of the stock standard approximately into the middle of the calibration range.

4.3.1.1. For the ERA standard (ERA SIM NUT (4.1.1.)), follow the manufacturer instructions to prepare the certified concentration standard from the ampule. Name the standard to indicate it's source, e.g. "NITRITE_ERA_P297-770."

Store refrigerated. Holding time 30 days.

4.3.1.2. If the concentration of the certified standard (4.3.1.1.) is near the mid-calibration range (0.10 mg N/L) or below, name the standard QCNI and use it on the run. If the concentration of the certified standard is too high, dilute it with DI water to prepare the QCNI standard. Into a 1 L flask containing approximately 500 mL DI water, pipette appropriate volume (V1) of the certified standard based on the formula below.

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$$M1V1 = M2V2,$$

Where,
M1 = Concentration of the certified standard - concentration can be obtained from the Certificate of Analysis
V1 = Volume of stock required
M2 = Concentration of working standard
V2 = Volume being prepared = 1.0 L

Bring to volume with DI water and invert 12 times.

Store in refrigerator. Holding time, 30 days.

4.3.2. Stock Nitrite Standard, 2000 mg N/L –

KNO2 (4.1.10.)	12.14 g
Chloroform (4.1.9.)	2 mL
DI Water (4.1.12.)	to 1000 mL

Pour the Potassium Nitrite (KNO2) in a glass beaker and place it in an oven for 24 hours at 105 °C. Dissolve 12.14 g of the dried Potassium Nitrite in approximately 800 mL of DI water contained in a 1 L volumetric flask. Add 2 mL of chloroform. Bring the volume to 1 L with DI water and invert 12 times.

Store in refrigerator. Holding time, 180 days.

4.3.3. Intermediate Stock Standard, 20.0 mg N/L –

Stock Nitrite Standard (4.3.2.)	10.0 mL
DI Water (4.1.12.)	to 1000 mL

Into a 1 L flask containing approximately 500 mL DI water, pipette 10.0 mL of the 2000 mg N/L nitrite stock standard. Bring to volume with DI water and invert 12 times.

Store in refrigerator. Holding time, 90 days.

4.3.4. Working Stock Standard, 0.20 mg N/L –

Intermediate Stock Standard (4.3.3.)	2.0 mL
DI Water (4.1.12.)	to 200 mL

Add 2.0 mL of the 20.0 mg N/L working stock standard to a 200 mL volumetric flask containing approximately 150 mL of DI water. Bring to volume with DI water and invert 12 times.

Store in refrigerator. Holding time, 30 days.

4.3.5. Continuous calibration verification standard, CCV, 0.10 mg N/L –

Intermediate Stock Standard (4.3.3.)	5 mL
DI Water (4.1.12.)	to 1000 mL

Add 5 mL of the 20.0 mg N/L working stock standard to a 1 L volumetric

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flask containing approximately 800 mL of DI water. Bring to volume with DI water and invert 12 times.

Store in refrigerator. Holding time, 30 days.

4.3.6. PQNI (PQL level standard), 0.005 mg N/L –

Intermediate Stock Standard (4.3.3.)	50 µL
DI Water (4.1.12.)	to 200 mL

Add 50 µL of the 20 mg N/L working stock standard to a 200 mL volumetric flask containing approximately 150 mL of DI water. Bring to volume with DI water and invert 12 times.

Store in refrigerator. Holding time, 30 days.

4.3.7. Working Nitrite Spike Standard, 4.0 mg N/L –

Stock Nitrite Standard (4.3.2.)	500 µL
DI Water (4.1.12.)	to 250 mL

Add 500 µL of the 2000 mg N/L nitrite standard to a 250 mL volumetric flask containing approx. 100 mL of DI water. Dilute to volume and invert 12 times.

Store in refrigerator. Holding time, 30 days.

5. SAMPLE HANDLING AND PRESERVATION

5.1. Samples are collected and stored on ice or in refrigerator at ≤ 6°C (not frozen).

5.2. Samples must be analyzed within 48 hours of collection time.

6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

Note: Samples containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 or AQ400 Discrete Analyzer.

6.1. Initial Preparation:

6.1.1. Remove standards and reagents from the refrigerator and check the expiration dates. Discard expired standards and/or reagents and prepare again if necessary.

6.1.2. Use 57-position sample tray for the runs on AQ2 instrument or use 80-position sample tray for runs on AQ400.

Note: Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook. Make sure to perform daily maintenance at the beginning of each day prior to analysis as described per Section 9.

6.2. Calibration:

6.2.1. The nitrite calibration is prepared from the 0.20 mg N/L working stock standard (4.3.4.) by auto-dilution on the instrument.

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Concentration (mg N/L)	Source
S1 – 0.0000	DI H2O
S94 – 0.2000	0.20 mg N/L Working Stock Standard (4.3.4) – Pour in Cup
S90 – 0.0048	Auto Dilution
S91 – 0.0100	Auto Dilution
S92 – 0.0400	Auto Dilution
S93 – 0.1000	Auto Dilution
S0 – 0.0000	DI H2O

6.2.2. The diluent for nitrite analysis is DI water.

6.3. Reagents, Run Controls, and Stock Standard Loading:

6.3.1. Label one AQ2/AQ400 reagent vessel and fill it with the NED color reagent (4.2.1.).

6.3.2. Reagent Location - The reagent tray is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. Place the NED color reagent (4.2.1.) in position 7.

6.3.3. Controls (CCV/check and CCB/blank) – The 0.1 mg N/L standard is used as the CCV standard (4.3.5.). The instrument blanks or CCBs are DI water. CCV and CCB standards should be run every 10 samples and at the beginning and end of each run. Previous experience shows that pouring CCV and CCB into AQ2/AQ400 reagent vessels may result in random failures. It is recommended, therefore, to pour CCV and CCB standards into 2 mL sample cups and place them in the 57-position sample tray (AQ2) or 80-position sample tray (AQ400) based on the run sequence.

6.4. Batch QC:

6.4.1. A laboratory fortified blank (LFB) and a sample either ran in duplicate and/or spiked in duplicate must be analyzed for every batch of 10 samples. Use the 4.0 mg N/L stock solution (4.3.7.) as the spiking solution. Add 150 µL to 10 mL of sample (or DI water in the case of an LFB) for a 0.060 mg N/L spike. The spike code in QC Manager is LO.

6.4.2. One method blank (MBLK) must be analyzed for every batch of 10 samples. DI water is used for the MBLK.

6.5. Analysis Setup/Sequence Setup:

Note: Software operation and user interface are exactly the same for all AQ2s and AQ400 instruments and steps below should be followed for all instruments.

6.5.1. Turn on the computer and start the software by double-clicking on the AQ icon on the screen. If running on AQ2-1, the username is '**Instrument 1**'. If using **AQ400**, the username is '**Instrument**'. The password for both the AQ2 and AQ400 is 'password'. Next turn on the AQ2/AQ400Analyzer (there are two switches, turn on the large switch on the right side behind the instrument - the small switch right next to it is for the chiller and is not

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used for this test).

6.5.2. Clean and refill the DI water reservoir and the waste containers. Replace all used reaction segments.

6.5.3. In the software: Go to the 'Settings' menu and select maintenance. Zero the reaction segment counter.

6.5.4. In the 'Initialise' tab under 'Maintenance and Setup', check that the tray size is set to 57, for the AQ2s, or 80, for the AQ400.

6.5.5. Go back to the 'settings' menu and select the daily start-up option.

6.5.6. Setup of the analytical run: Click on the scheduling icon on the left side of the screen. Select a free tray and **reagent set 2**. Name the run as follows:

AQ2-1: NOYYMMDDK1 (use 2 for the 2nd run that day, etc.)
AQ400-1: NOYYMMDDP(1)

Now the sequence screen appears. Leave lines one and two empty. Type the sample sequence in the 'Sample ID' column. Highlight the typed lines and select the NO2 test. The test should appear in the requested test column to the right of the sample ID's. Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

An example run sequence includes:

- **Calibration**
- **CCV** (4.3.5.)
- **CCB** (6.3.3.) DI water
- **MBLK** (6.4.2.) DI water
- **PQNI** (4.3.6.)
- **QCNI** (ALTQC, 4.3.1.)
- **LFB** (6.4.1.)
- **Batched samples**, with duplicate spikes (6.4.1.) for each batch
- **CCV**
- **CCB**

6.5.7. Pouring the analytical run - Cup positions #1 and #2 are reserved for the calibration standards. For NO2 the calibration standard (4.3.4.) is placed in position #2 (position #1 on the sample tray is unused). Now, start pouring the samples according to the cup position on the bench sheet.

6.5.8. Double-click on the 'Run' icon on the left side of the screen. A dialog box appears. Select the tray that contains the sequence to be analyzed. Click 'ok'. Another dialog box appears. Check the auto-standardize box and click 'ok' (if the analysis will use the previous calibration do not check the auto-standardize option.). Select 'ok' on the following dialog box.

6.5.9. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start. If not, the run will stop and display the reagent

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which has insufficient volume. Fill up the reagent and re-start the run according to 6.5.7. While the run is in progress, the method should be printed to be included in the analysis file. Go to the 'settings' menu and select the option 'Test Parameters'. Select the Test 'EPA353_2 Inst1, 2 or 3' and print.

6.5.10. If the run quality deteriorates (unacceptable calibration and/or QC results, high blanks, sample carry-over, etc.), the system should be washed with the alkaline EDTA rinse solution (4.2.2.) to remove any reagent deposits in the aspiration tube and cuvette. If carryover is expected or observed during a run, the Alkaline EDTA Rinse (4.2.2.) can be scheduled after every sample cup in the method parameters. The solution is placed in a reagent vessel in position 1 of the reagent tray and is called Control 1 in the sample sequence.

6.6. Post-Run Procedure:

6.6.1. Review water baseline values ("W" column) before accepting the results. Anomalous readings here may be indicative of the instrument performance problems. Any results associated with such readings should be questioned.

6.6.2. Click on the 'Data Review' menu located on the left side of the screen. Make any necessary changes to the sample list (dilutions, additional samples, etc.). Click on the 'Accept All' Icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the convention described in 6.5.6. Accepting the run in the AQ2/AQ400 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder at

\\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\rawdata\discreteanalyzers\AQ2-1\Nitrite\

or if using AQ400:

\\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\rawdata\discreteanalyzers\AQ400-1\Nitrite\

6.6.3. After the run has finished click on the 'Reporting' icon on the left side of the screen. Select the appropriate reporting format and print the run report in the chronological reporting format. It contains the calibration and all the data.

6.6.4. Convert the excel file into Raw data in the QC Manager with respect to the instrument used: The Instruments used are 'Nutrients: Seal AQ2 1' or 'Nutrients: Seal AQ400-1'.

6.6.5. Perform the following post-run cleaning: Select 'Extra Wash' in the 'Cuvette Functions' tab of the 'Maintenance' window. Perform the 'Extra Wash' again. After the extra washes, set the 'Auto Wash' number to 5. Run three sets of auto washes, for a total of 15 replicates.

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6.7. Quantitation/Calculations:

6.7.1. The instrument will give the analysis results for Nitrite in mg N/L.

6.7.2. The QC Manager calculates relative percent differences (RPDs) for duplicate samples as well as recoveries for QC standards. For general calculations including RPDs, see Section 5.9 of the FDEP Quality Manual. Once calculated, the results are uploaded into LIMS.

6.7.3. The high calibration standard is prepared according to section 4.3.4. The other standards used in the linear calibration are auto-diluted by the instrument from the high standard. The calibration consists of five standards and a calibration blank. The correlation coefficient for the calibration and the % error of the standards are calculated by the instrument software.

7. DATA ARCHIVAL/VALIDATION

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test(s) (W-NO2, W-NO2-F), filename/s (NOYYMMDDx# (where x is refers to the instrument code (see section 6.5.6.), and # indicates the 1st, 2nd etc. run of the day), and the initials of the analyst. If nitrate by calculation was performed as well, the test W-NO3 is added to the label.

7.2. Each report folder should contain the following items - the run report(s), bench sheet(s), reagents and standard logsheet, the backlog containing the batching documentation, NU-PIPETTE form, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. If nitrate by calculation was performed a copy of the nitrate by calculation excel sheet (see DEP SOP NU-066, section 9) should be included. If sample dilutions were prepared, the dilutions recipes and the pipettes used are documented on the NU-PIPETTE form.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. AQ2/AQ400 instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's Nitrite subfolder located at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\rawdata\discreteanalyzers\AQ2-1\Nitrite\

for AQ2, or for AQ400 \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\rawdata\discreteanalyzers\AQ400-1\Nitrite\

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\FLDEP1\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

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8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE DEP SOPS NU-038 AND NU-043)

8.1. For method performance and acceptance criteria, for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to the following DEP SOPs: NU-038 and NU-043.

8.2. The 0.1 mg N/L CCV standard (4.3.5.) and CCB will be run every 10 samples and at the beginning and end of each run. Acceptance limits for the recovery of the CCV are 90 –110%. Acceptance limits for the CCB and MDL are < 0.002 and > -0.002 mg N/L. For convenience, the QCNI standard (8.3) may be used as the CCV.

8.3. The 0.1 mg N/L QCNI standard (4.3.1.) is prepared from a different source than the calibration standards. It serves as the ICV (initial calibration verification) standard and must be run after the calibration standards to verify the calibration. Acceptance limits for the recovery of the QCNI are 90 – 110%.

8.4. The PQL level standard (PQNI, 4.3.6.) is run on every analytical run and serves to verify the instrument performance at the low concentration range and as the ongoing MDL and PQL verification spike. Acceptance limits for the recovery of the PQNI are 70 – 130%.

8.5. An MBLK, an LFB, a matrix spike, and a sample or matrix spike duplicate are analyzed for every batch of 10 samples. Acceptance limits for the MBLK are < 0.002 and > - 0.002 mg N/L. Acceptance limits for the recovery of the LFB and spikes are 90 – 110%. The sample/matrix spike reproducibility should be less than 20% RPD for samples above the PQL.

8.6. Linear Calibration Range (LCR): See FDEP Quality Manual Appendix B, Section 7.0., 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear calibration must be equal to or greater than 0.995. Acceptance limits for the percent error of calibration standards above the PQL are ±10%. Acceptance limits for the percent error of the PQL level standard (0.048 mg N/L) are ±30%. The acceptance limits for the blank calibration point results are below the MDL and above the negative value of the MDL (-MDL < blank < MDL).

8.7. Every day samples are analyzed a new calibration is performed at the beginning of the analysis and the LCR must be verified. Consecutive runs on one day may use the same calibration.

9. MAINTENANCE AND TROUBLESHOOTING

Note: For both AQ2 and AQ400 instruments, the steps below must be followed for daily maintenance (exception is the "adjustment of aspiration step 9.15.)

9.1. Prepare a 1% v/v bleach solution, a beaker of DI water, and empty and rinse the DI water reservoir at least three times. Refill it to the top.

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9.2. Empty the instrument rinse waste container.

9.3. Place the instrument probe in the bleach solution.

9.4. In the SEAL AQ2/AQ400 Program, go to 'Settings' then 'Maintenance.'

9.5. In 'Initialize,' press 'Zero Segments.' **Do not press 'OK' yet.**

9.5.1. Check to see if there are enough reaction segments from the day before to do maintenance. 2-3 unused is enough. Then press 'Cancel.' The sampler probe will go to the first clean segment instead of Segment 1, spot 1.

9.5.2. If there are not enough clean segments, replace the segments. Double tap clean segments on the bench to knock out excess manufacturing particles, then place in the instrument. Press 'OK' to reset the used segments. The sampler probe will go to Segment 1, spot 1.

9.6. Go to 'Diluter,' set the number of primes to 10, then press 'Start Primes.' Press 'Start Primes' twice more for a total of 30 primes in the bleach solution.

9.7. Go to 'Cuvette Functions,' set the drop-down number to 5, then press 'Auto Wash.' Press 'Auto Wash' twice more for a total of 15 washes in the bleach solution.

9.8. Transfer the instrument probe from the bleach cleaning solution to the beaker of DI water.

9.8.1. Repeat the 'Primes' three times for a total of 30 primes.

9.8.2. Repeat the 'Auto Wash' three times for a total of 15 washes.

9.9. Transfer the instrument probe to the DI water reservoir.

9.9.1. Repeat the 'Primes' five times for a total of 50 primes.

9.9.2. Repeat the 'Auto Wash' five times for a total of 25 washes.

9.10. Clean the aspiration bath with a Q-tip or cleaning swap.

9.10.1. The particles from manufacturing can get caught in the cuvette. Daily cleaning of the aspiration bath helps prevent this from occurring.

9.11. In a reagent wedge in position 1, place the alkaline EDTA rinse (4.2.2.). In 'Cuvette Functions,' press 'Extra Wash.'

9.11.1. Watch the glass probe cleaner. A water vortex should be visible when the probe is being washed and liquid should not drip out.

9.11.2. Contamination between samples and reagents can occur if the probe cleaner is not operating properly. Liquid drips out of the glass probe cleaner when the probe creates a wider hole at the bottom of the glass (will look oval). This can occur over time or if the probe is bent. Replace the probe cleaner and probe if necessary.

9.11.3. Watch the syringe piston to see if there are excess bubbles dragging from the top. A worn syringe piston can interfere with the calibration auto-

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dilution and analysis. Replace if necessary.

9.12. After 'Extra Wash' is done, set the number of 'Auto Wash' to 5. Then press 'Auto Wash.'

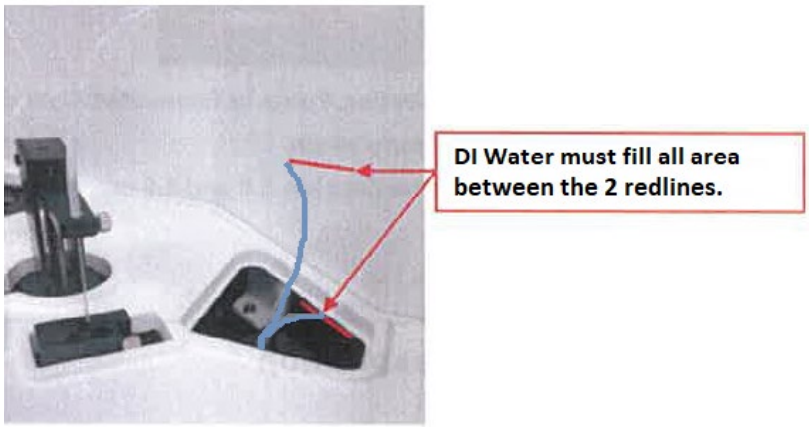
9.12.1. Make sure the aspiration bath is filling and emptying each time. Change the two peristaltic tubing going to the aspiration bath if it is not.

9.13. After 'Auto Wash' is done, press 'Extra Wash' again.

9.14. After the second 'Extra Wash' is done, press the 'Auto Wash' again.

9.15. To adjust the aspiration, remove the cover from the top of the cuvette/lamp. Press 'Test Aspiration' and observe where the water line begins. For **AQ2**, it should be around 2 inches out of the cuvette. Repeat 'Test Aspiration' 2 more times, then check that the 4th, 5th, and 6th repetition are in the same spot.

For **AQ400** see diagram below:



AQ400 – DI water must flow completely through the cuvette and fill the areas between the 2 redlines shown above, approximately 1-2 inches on either side of cuvette (see AQ400 Operation Manual page 30 for reference).

9.15.1. To make the water line come out of the cuvette further, increase the number of steps. Decrease the number of steps if it is too far out. Then repeat the above test.

9.15.2. If the water line is not around the same location in each repetition, random errors may occur in analysis. Replace the peristaltic tubing on the pump located next to the lamp.

9.16. In 'Initialise,' press 'Zero Segments,' and replace all used segments. Then press 'OK.'

9.17. Close the maintenance window then go to 'Settings' → 'Daily Startup' → 'Continue.' Write down the highest voltage and the corresponding filter in the maintenance book.

9.17.1. For the AQ2, the highest voltage filter should remain the same for each

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instrument. A drastic change in voltage from day to day indicates a lamp going bad or an electrical issue.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

10.1. Review the Laboratory Health and Safety Plan (available at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

10.2. Safety glasses, gloves and lab coats must be worn at all times while handling samples and reagents.

10.3. Read the MSDS information for safe reagent and sample handling.

10.4. Instrument and sample waste are flushed down the drain with plenty of water.

11. REFERENCES/DEFINITIONS

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

11.1. EPA method 353.2, Revision 2.0, August 1993.

11.2. Seal Analytical AQ2/AQ400 method EPA-137-A Rev.1.

11.3. AQ2 Operator Handbook and AQ Software guide.

11.4. AQ400 Discrete Multi-Chemistry Analyzer Operation Manual

11.5. Quality Manual, Bureau of Laboratories, DEP.

11.6. Laboratory Health and Safety Plan.

11.7. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

11.8. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.9. DEP SOP NU-041, Reagent Preparation Procedure.

11.10. DEP SOP NU-042, Standard Preparation Procedure.

11.11. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

11.12. DEP SOP NU-066, Determination of Nitrate and Nitrite in Water Using the Lachat QuickChem 8500 Autoanalyzer

11.13. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

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Appendix of Significant Changes

12/01/2010	Changed MDL level to 0.002 mg N/L and PQL to 0.005 mg N/L. Made changes to reflect the use of all three Seal AQ2 instruments.
11/29/2011	A section was added to list the chemicals used for the Nitrite analysis.
11/19/2012	A troubleshooting section was added to help restore/maintain run quality using an alkaline/EDTA wash procedure.
11/05/2015	Data archival information was clarified in Section 7.
12/27/2018	Section 4 is modified to include a reference to new storage location for certificates of analysis. In Section 8, added a reference to use of the PQL level standard for the ongoing MDL and PQL verification data collection. Section 10 waste disposal procedure is clarified.
02/13/2020	Added control limits for the blank calibration standard.
11/25/2020	Added clarifications to section 8 on use of the ICV.
03/24/2021	Clarified Section 1. Revised Sections 3, 4, and 6 to add information tables. Revised 4.3.1. to include preparation of QCNI for the ERA certified standards.
12/19/2021	Revised section 4.3.1. and added 4.3.5. for new procedure of preparation of the QCNI and CCV standards.
04/21/2023	Added instruments AQ2-1 and AQ400-1. Retired instrument AQ2-2. Added maintenance section (section 9).

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